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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/751,235	01/02/2004	Dean DellaPenna	MSU-08604	3881

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EXAMINER	
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ART UNIT	PAPER NUMBER
1638	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/751,235	DELLAPENNA ET AL.	
	Examiner	Art Unit	
	Cathy K. Worley	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 May 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8,10-17 and 21-37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-17 and 21-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on May 22, 2007, has been entered.

2. Claims 9 and 18-20 have been canceled.

Claims 33-37 have been newly added are drawn to the elected invention. Claims 1-8, 10-17, and 21-37 are pending and are examined in the present Office Action.

Specification

3. The title remains objected to for not being descriptive of the elected invention.

Claim Objections

4. Claims 22 and 33 are objected to because of the following informalities:

- In claim 22, the recitation is technically incorrect because a plant cannot "comprise" more than one species. The Applicant is advised to amend the claim to replace "comprises one or more of the following:" with - - is selected from the group consisting of - - .
- In claim 33, the claim does not end with a period, and the claim is also technically incorrect because a carotene cannot "comprise" both lutein and α.-carotene.

Appropriate correction is requested.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 33 and 35-37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 33 includes "(β-ε.-carotene-3'-ol)", and it is unclear what the significance of this parenthetical recitation is. Does this mean that the α.-carotene

recited immediately prior must be β-ε.-carotene-3'-ol? If so, this is incorrect because the α.-carotene would have to be modified. The Applicant is advised to amend the claim to remove the parentheses and to clearly indicate what compounds are encompassed by the claim.

Claim 35 recites the limitation "said transgenic plant" in line 1 of part "c)". There is insufficient antecedent basis for this limitation in the claim.

Claims 36 and 37 recite the limitation "The plant tissue of Claim 34" in line 1. There is insufficient antecedent basis for this limitation in the claim.

The term "desired" in claim 37 is a relative term which renders the claim indefinite. The term "desired" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. This recitation implies that in some cases expression is desired and in other cases expression is not desired, and there is no teaching of how one would know when it would be desired or not.

6. Claims 1-8, 11-17, and 21-37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s),

at the time the application was filed, had possession of the claimed invention. All dependent claims are included in the rejection.

The claims are broadly drawn to expression vectors, nucleic acids, transgenic plants and seeds, and methods comprising a nucleic acid sequence encoding a polypeptide at least 72% identical to SEQ ID NO:4 and having monoxygenase P450 activity.

The Applicants describe the polypeptide of SEQ ID NO:4 which is encoded by SEQ ID NO:5 (see sequence listing). The Applicants speculate that the enzymatic activity encoded by SEQ ID NO:5 is ε-ring hydroxylase or β-ring hydroxylase activity from a cytochrome P450 monooxygenase (see page 102 lines 3-5, pages 103-104 Example 5, and page 101 lines 3-5). The specification describes motifs/domains that are known in the art to correspond to an oxygen binding pocket, SEQ ID NO:12, a transmembrane domain, SEQ ID NO:10, a cysteine/heme-binding motif, SEQ ID NO:14, and a chloroplast targeting peptide, SEQ ID NO:11 (see pages 102-103), but there are no working examples where these activities are proven. The only proven functional activity is the ability of SEQ ID NO:5 (which encodes SEQ ID NO:4) to complement the *lut1* mutation in *Arabidopsis*. .

The Applicants do not describe the motifs and domains that are sufficient for providing functional activity that will complement the *lut1* mutation in *Arabidopsis*. They do not describe a sufficient number of representative species of the large genus being claimed.

The essential feature of the instant invention is the ability to complement the *lut1* mutation in *Arabidopsis* through an activity that produces zeinxanthin.

The Federal Circuit has recently clarified the application of the written description requirement to inventions in the field of biotechnology. The court stated that, "A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus." See *University of California v. Eli Lilly and Co.*, 119 F. 3d 1559; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

Claims 1, 15, 21, 25-32, 34, and 35 recite "a nucleic acid sequence encoding a polypeptide at least 72% identical to SEQ ID NO:4" in conjunction with monoxygenase P450 activity. SEQ ID NO:4 identifies a polypeptide consisting of 539 amino acid residues, therefore a polypeptide that is at least 72% identical to SEQ ID NO:4 can have 150 amino acid substitutions. Given that there are 20 different amino acids, the genus of molecules that can have any amino acid residue at 150 different positions within the polypeptide encompasses 20^{150} molecules which is 1.4×10^{195} molecules. Furthermore, each of these multitudes of polypeptides can be encoded by any nucleic acid molecule having the necessary codons, so the genus of nucleic acid molecules encompassed by these claims is even larger than the genus of polypeptides. There was only one nucleic acid molecule (SEQ ID NO:5) shown to

have functional activity by complementing a mutant phenotype in *Arabidopsis* (see pages 101-102, Example 3, in particular). Given the multitudes of molecules encompassed by the claims and only one shown to have any functional activity, this is not a representative number of species for the large genus being claimed.

The specification speculates that the enzymatic activity encoded by SEQ ID NO:5 is ε-ring hydroxylase or β-ring hydroxylase activity from a cytochrome P450 monooxygenase (see page 102 lines 3-5, pages 103-104 Example 5, and page 101 lines 3-5, in particular). The specification describes motifs/domains that are known in the art to correspond to an oxygen binding pocket, SEQ ID NO:12, a transmembrane domain, SEQ ID NO:10, a cysteine/heme-binding motif, SEQ ID NO:14, and a chloroplast targeting peptide, SEQ ID NO:11 (see pages 102-103, in particular), but there are no working examples where these activities are proven. The only proven functional activity is the ability of SEQ ID NO:5 (which encodes SEQ ID NO:4) to complement the *lut1* mutation in *Arabidopsis*. However, the specification does not describe any specific structural features that correspond to the functional activity of being able to complement the *lut1* mutation.

The Applicants fail to describe a representative number of polypeptides having at least 72% identity to SEQ ID NO:4 and having the ability to complement the *lut1* mutation in *Arabidopsis* through an activity that produces zeinxanthin. The Applicants only describe SEQ ID NO:4. Furthermore, the Applicants fail to describe structural features known to be sufficient for producing zeinxanthin in

Arabidopsis and common to members of the claimed genus of polypeptides. Hence, Applicants fail to meet either prong of the two-prong test set forth by *Eli Lilly*. Furthermore, given the lack of description of the necessary elements essential for the activity of producing zeinxanthin in *Arabidopsis* and complementing the *lut1* mutation, it remains unclear what features identify polypeptides capable of such activity. Since the genus of polypeptides has not been described by specific structural features, the specification fails to provide an adequate written description to support the breadth of the claims.

Nucleic acids that encode polypeptides with at least 72% identity to SEQ ID NO:4 encompass a large number of molecules, many of which would not have the necessary function of producing an enzyme in a plant that is capable of producing zeinxanthin or the function of complementing the *lut1* mutation in *Arabidopsis*, and most of which were not in the Applicant's possession at the time of filing. The Applicants have not reduced a single molecule to practice in an experiment that demonstrates an affect on carotenoids in a wild-type plant. Accordingly, the specification fails to provide an adequate written description to support the genus of nucleic acids that encode polypeptides with at least 72% identity to SEQ ID NO:4 as set forth in the claims. (See Written Description guidelines published in the Federal Register/Vol. 66, No. 4/Friday, January 5, 2001/Notices: p. 1099-1111).

Given the large genus of molecules encompassed by the claims, and given the lack of description of structural features corresponding to the function of producing

zeinxanthin or the function of complementing the *lut1* mutation in *Arabidopsis*, the written description requirements have not been met.

The Applicant argues that the attached Declaration of Dr. Dean DellaPenna demonstrates that a person of ordinary skill in the art is able to make and identify a nucleic acid sequence encoding a polypeptide that is at least 78% identical to SEQ ID NO:4 (see remarks directed to the written description rejection in the response filed on May 22, 2007). This is not persuasive, however, because the Office did not receive any Declaration in the papers received on May 22, 2007.

The Applicant argues that they have provided internal structural features of SEQ ID NO:4, and described how it was used to identify a larger genus of molecules. This is not persuasive, however, because the structural features merely identify domains that are common to other cytochrome P450 proteins. None of these other proteins have been shown to have the function of complementing the *lut1* mutation in *Arabidopsis*. There is no evidence that the particular domains described in the instant application are sufficient for the specific function of producing zeinxanthin; polypeptides with those domains could have completely different substrates and functions *in vivo*.

For the reasons explained above, claims 1-8, 11-17, and 21-37 are rejected under 35 USC 112,first paragraph for lack of written description.

7. Claims 1-8, 10-17, and 21-37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 1-8, 10-17, and 21-37 are broadly drawn to expression vectors, nucleic acids, transgenic plants and seeds, and methods comprising a nucleic acid sequence encoding a polypeptide at least 72% identical to SEQ ID NO:4 and having monoxygenase P450 activity.

The nature of the invention is molecular biological approaches for using a nucleic acid discovered by complementing a mutant.

The specification discloses that a nucleic acid comprising SEQ ID NO:5 was identified by its ability to complement the *lut1* mutation in *Arabidopsis* (see page 102 lines 6-9 and Figure 19a). This nucleic acid encodes the amino acids identified as SEQ ID NO:4 (see Figure 19a). A subsequence of SEQ ID NO:4 is identified as SEQ ID NO:1 (see Figure 18). The specification discloses that bioinformatics analyses suggests the polypeptide of SEQ ID NO:4 is a cytochrome P450 enzyme and comprises an oxygen binding pocket consensus sequence (SEQ ID NO:12), a heme-binding cysteine motif (SEQ ID NO:14), a chloroplast targeting peptide (SEQ ID NO:11), and a transmembrane domain (SEQ ID NO:10), (see pages 102-103 and Figure 22).

The specification does not disclose any enzyme assays showing that the protein encoded by SEQ ID NO:5 has a specific enzymatic function. Transformation of the *lut1* mutant *Arabidopsis* plant with SEQ ID NO:5 complements the mutant phenotype and therefore, either directly or indirectly, provides ε-ring hydroxylase and β-ring hydroxylase activity (see page 103-104 and Figure 17). Furthermore, subsequent experimental work was unsuccessful in providing an assay for enzymatic function (see Tian et al PNAS (2004) Vol. 101, pp. 402-407). Tian et al teach that initial attempts to express and assay LUT1 protein in yeast were unsuccessful (see Tian et al, page 405, left column), and expression in bacteria is highly unlikely to work given the problems of expression eukaryotic membrane proteins in prokaryotic systems (see Hannig et al TIBTECH (1998) Vol. 16, "focus", see second-to-last page, right column). Therefore, one of skill in the art would not know how to use the nucleic acids and vectors for prokaryotic or yeast expression (claims 11 and 14 are specifically not enabled for these reasons).

The instant application speculates that SEQ ID NO:5 encodes a cytochrome P450 enzyme with ε-ring hydroxylase and β-ring hydroxylase activity that is involved in carotenoid biosynthesis, however, even if this hypothesis is true, multiple enzymes are involved in this pathway, and it is highly unpredictable what phenotype would result from overexpression of only one of the enzymes involved. The prior art teaches that metabolic engineering of biosynthetic pathways is highly unpredictable (see Stephanopoulos et al TIBTECH (1993), Vol. 11, pp. 392- 396). It

is possible the required enzymes may have to be present in stoichiometric quantities, or there could be feedback regulation mechanisms that are complex. It would require undue experimentation on the part of one of skill in the art to determine the results of expressing SEQ ID NO:5 in a plant, and to elucidate what other steps (if any) would be required to generate a useful plant.

Given this unpredictability and given that the specification in the instant application has not provided any working examples of expression of SEQ ID NO:5 in a healthy wild-type plant to demonstrate there is an effect on carotenoid metabolism (other than complementing a mutant which is deficient in the identical enzyme), one of skill in the art would not know how to use the claimed expression vectors, nucleic acids, transgenic plants and seeds, and the methods recited in claims 28-32 are not enabled.

POTENTIAL SCOPE OF ENABLEMENT

Even if the Applicant can provide support for a use of SEQ ID NO:5, the enablement would not be extended to the entire genus of molecules encompassed by these claims. The claims encompass nucleic acids encoding polypeptides with as little as 72% identity to SEQ ID NO:4, and one of skill in the art would not know how to use any such nucleic acids. The specification does not disclose any nucleic acid other than SEQ ID NO:5 that encodes a polypeptide at least 72% identical to SEQ ID NO:4 that has been shown to have the function of producing zeinxanthin and the function of complementing the *lut1* mutation in *Arabidopsis*, and there are

multitudes of nucleic acids encompassed by this recitation as discussed above in the written description rejection. Even if there were some guidance on how to use a nucleic acid encoding ε-ring hydroxylase and β-ring hydroxylase activity that is involved in carotenoid biosynthesis, there is no guarantee that a polypeptide with as little as 72% identity to SEQ ID NO:4 would have monoxygenase P450 activity. The specification has not provided any working example to demonstrate that a polypeptide having as little as 72% identity and comprising SEQ ID NO:1, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:14 would be capable of catalyzing any reaction at all. These domains, put together, have not been shown to be sufficient for enzymatic activity or the desired carotenoid biosynthesis function.

Given the breadth of the claims, the unpredictability in the art, and the lack of working examples, it would require undue experimentation on the part of one of skill in the art to make and use the invention as claimed.

The Applicant argues that the declaration by the inventor describes expression of a rice homolog of LUT1 in E. Coli. This is not persuasive, however, because the Office did not receive any Declaration in the papers received on May 22, 2007.

The Applicant argues that the specification describes a prokaryotic expression vector and a yeast expression vector, and that the Tian paper was submitted before the present application was filed. This is not persuasive, because the Tian paper is the only publication in which expression of LUT1 in yeast was

attempted. There are no publications showing successful expression of LUT1 in a prokaryote, in a yeast, or in a wild-type plant. The only publication where expression in yeast was attempted, discloses that they were not able to express a functional enzyme in yeast (see Tian et al).

The Applicant argues that the Examiner relied on outdated papers published in 1993 and 1998 to demonstrate unpredictability. This is not persuasive, however, because, between 1993/1998 and the time of filing (2004), there was no leap forward in methods of expressing LUT1 in yeast or methods of expressing membrane proteins in prokaryotes taught in the art. The instant specification has not taught any specific strategies to overcome the difficulties of attempting to express a membrane protein in a prokaryote. Furthermore, there is no evidence that the prophetic method for expressing LUT1 in yeast that is taught in the instant application is different in any way from the method attempted by Tian et al which was not successful.

It is noted that the Applicant has not provided any arguments about the unpredictability of expressing one component of a metabolic pathway in an attempt to increase the yield of a product of the pathway. This is the main issue with regard to enablement of the instant invention. In the instant invention, the Applicant is attempting to modify carotenoid biosynthesis by expressing one enzyme. However, multiple enzymes are involved in this pathway, and it is highly unpredictable what phenotype would result from overexpression of only one of the enzymes involved.

The Examiner emphasizes that the prior art teaches that metabolic engineering of biosynthetic pathways is highly unpredictable (see Stephanopoulos et al TIBTECH (1993), Vol. 11, pp. 392- 396).

For the reasons stated above, it would require undue experimentation on the part of one of skill in the art to determine the results of expressing SEQ ID NO:5 in a plant, and to elucidate what other steps (if any) would be required to generate a useful plant.

8. All claims remain rejected.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Cathy K. Worley whose telephone number is (571) 272-8784. The examiner has a variable schedule but can normally be reached on M-F 10:00 - 4:00 with variable hours before 10:00 and after 4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg, can be reached on (571) 272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

CKW

8/4/07

/Medina A. Ibrahim/

Primary Examiner
Art Unit 1638